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UPTAKE AND ACTION OF A DISULPHONIC STILBENE (SITS) IN THE PERFUSED GUINEA-PIG LIVER: A COMPARISON WITH BROMSULPHTHALEIN

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SUMMARY

1. Livers were perfused with a Krebs–Ringer bicarbonate buffer in a single-pass perfusion system. Bile secretion was maintained by infusion of secretin. 4-Acetamido-4′-isothiocyano-2,2′-stilbene disulphonic acid (SITS) was added to the perfusate to give concentrations ranging between 5×10^{-6} and 10^{-4} M.

2. SITS was extracted from the perfusate by the liver (V, 0·15 μmol/min per g liver; K_m $8{\cdot}6 \times 10^{-5}$ M) and excreted in bile in a modified form (bile/plasma ratio: 50–170; maximum rate of excretion: 25 nmol/min per g liver wet wt).

3. The rates of uptake and excretion of bromsulphthalein (BSP) were similar to those for SITS, with the exception that the affinity of BSP for hepatic uptake was greater (K_m $1{\cdot}8 \times 10^{-5}$ M).

4. Both SITS and BSP decreased the rate of bile flow. A 50% reduction in bile flow was attained in each case at an estimated drug content of the liver of 1·5 μmol/g wet wt.

5. Unlike other cells the hepatocyte appears to be readily penetrated by SITS, and it is suggested that SITS inhibits bile secretion by inhibiting an intracellular mechanism which could be mitochondrial in location.

INTRODUCTION

Since Knauf & Rothstein (1971) first demonstrated the inhibitory effects of SITS (4-acetamido-4′-isothiocyano-2,2′-stilbene disulphonic acid) on anion transport in red blood cells, this substance has been used to investigate anion transport in a variety of epithelia including turtle bladder, gall-bladder, renal proximal tubule and pancreas (Brodsky, Durham & Ehrenspeck, 1979; Heintze, Olles, Petersen & Wood, 1978; Green, Greenwood & Giebisch, 1980; Hutson & Scratcherd, 1980). Apart from a report by Marinetti & Gray (1967) in which the use of SITS as a fluorescent marker for the liver cell membrane was investigated, the effects of SITS on the liver have not been explored.

Preliminary studies of ours employing an isolated liver perfusion system showed that SITS could inhibit bile secretion and that the liver was capable of extracting SITS from the perfusate and excreting it into bile in large amounts (Borjian &

Rutishauser, 1981; Rutishauser, 1981). Detailed analysis of the transport of SITS by the liver and the effects of SITS on bile secretion is presented here. The results are compared with those obtained in the same perfusion system for another organic anion, bromsulphthalein (BSP), whose hepatic transport and metabolism have been studied in detail (Reichen & Paumgartner, 1980).

METHODS

Animals. Eighteen male varie-coloured guinea-pigs (*Cavia porcellus*) of body weight 390 ± 36 g (mean ± S.D. of an observation) were used. Food was withheld for 18 h before each experiment but water was allowed *ad libitum*.

Liver perfusion. Animals were anaesthetized intraperitoneally with urethane (25% solution: 7·5 ml/kg body wt; two-thirds as an initial dose). The cystic duct was ligated and the gall-bladder was drained. The bile duct was cannulated with a short length of polythene tubing (14 cm; internal diameter 1·14 mm; external diameter 1·57 mm). The portal vein was cannulated with a short length of wide-bore tubing (internal diameter 3 mm) which was tapered over the last 1½ cm to an external diameter of 2 mm and an internal diameter of 1·2 mm. Immediately before cannulation 0·2 ml heparin (5000 i.u./ml) was injected intraportally. The hepatic artery was cut but not perfused. The inferior vena cava was cut both above and below the liver and free drainage of effluent perfusate through these vessels was ensured. The liver was completely excised and transferred to the perfusion circuit, which was housed in a thermostatically controlled cabinet. A single-pass perfusion system was used. Livers were perfused at the rate of 50 ml/min (≡ 3·5–5·5 ml/min per g liver) with a Krebs–Ringer bicarbonate buffer for 2–3 h at 38 °C. The rate of flow of perfusate through the liver was checked at 5 min intervals and maintained at 50 ml/min by adjusting the perfusion pressure. Perfusate composition (mM) was: NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 5; gassed with 95% O_2–5% CO_2. Unless otherwise stated secretin was infused throughout each experiment (10 mu./min). Secretin (Karolinska Institute, Sweden) was dissolved in 0·9% sodium chloride containing 1% bovine serum albumin (Sigma) and infused via a motor-driven syringe at the rate of 76 µl/min.

The characteristics of bile secretion by the liver, in this perfusion system, have been described elsewhere (Borjian & Rutishauser, 1982).

Experimental protocol. After a satisfactory perfusion had been established the infusion of secretin was begun and livers were perfused for a control period of 45 min. The perfusate was then switched to one containing either SITS (BDH Chemicals Ltd, England) or BSP (Koch-Light Laboratories Ltd, England) and perfusion was continued for another 45 min. Finally perfusion with the control perfusate was resumed for a further 45 min. Ten paired samples of perfusate and of the effluent draining from the liver were collected at 5 min intervals, beginning with a control pair obtained 2½ min before the changeover of perfusate from control to that containing SITS or BSP. The lag between changeover time and the arrival of the new perfusate at the liver was 40–60 s. Bile was collected under paraffin oil throughout each experiment as serial 15 min samples whose volume was estimated by weighing (assuming specific gravity of bile = 1·0). Precautions were taken at all times to minimize the likelihood of *trans*-SITS being converted into its *cis* isomer: the SITS solution was prepared only 15 min before it was used; the perfusion circuit was illuminated almost exclusively by fluorescent light which itself did not cause appreciable deterioration of the SITS during the experiment; all samples of perfusate and effluent were protected from daylight and analysed almost immediately for their SITS content. Bile samples were analysed for their SITS content within 2–3 h of collection and then frozen for later analysis by chromatography. A check was made on the extent of deterioration by exposing standard solutions to the same environmental conditions.

Chemical analyses

SITS in perfusate. The absorbance of the perfusate and effluent samples was measured spectrophotometrically at 340 nm after appropriate dilution with water (Marinetti & Gray, 1967). The wave-length of maximum absorption was the same for perfusate and effluent, suggesting that SITS had not undergone appreciable change in its passage through the hepatic circulation. The

perfusate solution as prepared was used as the reference standard for calculating the concentration of SITS in the effluent.

SITS in bile. The absorption spectrum of bile obtained during the infusion of SITS was almost identical to that for a pure solution of SITS in its *trans* form. Maximum absorbance was attained in each case at 335–340 nm. On the basis of these findings the concentration of SITS in bile was determined spectrophotometrically at 340 nm after appropriate dilution of the sample with water. The absorbance of the pre-SITS bile sample was also measured in order to correct for the slight absorbance at this wave-length (1–2 %) contributed by other components in bile. The net absorbance of the bile samples was then compared with that of identically diluted standard solutions of SITS in water (2·5, 5·0 and 10·0 mM).

As a check on the validity of this method the measured concentration of SITS in bile was compared with the anion deficit in the bile calculated as the difference between the summed concentrations of sodium and potassium, and the summed concentrations of chloride and bicarbonate. A significant correlation existed ($r = 0{\cdot}87$; $n = 36$; $P < 0{\cdot}001$). The relationship between the calculated concentration of SITS (y) and the measured concentration of SITS (x) is described by the linear regression $y = 1{\cdot}25x + 0{\cdot}09$. The analytical method may therefore underestimate the true concentration of SITS in bile by about 20 %. The conversion of small amounts of SITS from its *trans* to its *cis* form could contribute to this as *cis*-SITS has an absorption maximum of 320 rather than 340 nm and a smaller extinction coefficient (Marinetti & Gray, 1967). In three experiments the absorbances of bile and standard SITS were also measured at 293 nm, the wave-length at which the absorption spectra for *trans*- and *cis*-SITS intersect. The estimated contributions made by *cis*-SITS were 2, 13 and 17 % respectively.

Chromatography of SITS in bile. Bile (10 μl) or standard SITS solution (10 mM) was applied as a spot to 20 cm × 20 cm thin-layer plates of thickness 500 μm prepared from Kiesel-gel (Camag) containing an ultraviolet indicator. The plates were developed in a solvent system consisting of butanol/acetic acid/water (2:1:1, by volume). The spots were visualized under ultraviolet light, SITS giving a vivid blue fluorescence. The silica gel from circumscribed areas was carefully removed and eluted with 4·0 ml of distilled water. The absorbance of the eluates was determined as before at 340 nm and corrected for the absorbance contributed by a blank area of the plate. The absorbance contributed by any one area was then calculated as a percentage of the total absorbances of all the areas eluted for that sample.

In order to check that the mobility of SITS was not affected by other substances present in bile, such as protein, SITS was added to samples of bile and then chromatographed.

BSP in perfusate and bile. The absorbance of the solution was determined spectrophotometrically at 580 nm after appropriate dilution and the addition of sodium hydroxide.

Ionic analyses. Sodium and potassium concentrations were determined by flame photometry (Corning-EEL 450); bicarbonate by a manometric technique (Natelson microgasometer); and chloride by electrometric titration (Corning-EEL 920). SITS interfered with the determination of chloride in that the conductivity changes signalling the end of the titration occurred before all the chloride present had been precipitated. If the same sample was retitrated, a further flow of current occurred and more chloride was precipitated. By repeatedly titrating the same sample and summing all the counts it was possible to precipitate all the chloride present. The validity of this method was checked using chloride solutions of known concentration to which SITS was added at concentrations similar to those in bile.

Calculations

SITS and BSP content of liver. This was estimated at any point in time after the start of infusion as the accumulated difference between the uptake of each drug into the liver and its excretion into bile. In practice this was achieved by determining the area between the uptake and excretion curves.

Percentage bile flow. Bile flow in each experiment was normalized by taking flow in the 30–45 min period after the start of the perfusion as equal to 100 % and expressing all flows before and after as a percentage of this value.

Percentage change in bile flow. This is the difference in percentage bile flow, at a specified period, as compared with that observed in control experiments over the same period. For example, using the data from Table 1, the percentage change in bile flow during the infusion of 4×10^{-5} M-BSP is $100 - (40{\cdot}1/98{\cdot}4)$.

Net changes in ionic composition of bile. These were calculated as:

$$\text{Net change in } [X]_{75\text{–}90\,\text{min}} = [X]_{75\text{–}90\,\text{min}} - \left(\frac{[X]_{30\text{–}45\,min} + [X]_{120\text{–}135\,min}}{2}\right),$$

where [X] is the concentration of the ion and the subscripts refer to the period of sampling.

Statistical analyses. Differences between mean values were assessed using Student's t test. Regression lines were calculated using the method of least squares.

RESULTS

Hepatic uptake

The pattern of uptake of SITS into the liver during 45 min of perfusion with solutions containing different concentrations of SITS is shown in Fig. 1. The 'initial' rate of uptake is a function of the rate of entry of SITS into the liver. The 'steady-state' rate of uptake represents the equilibrium situation where uptake is balanced by the rate of disposal of the transported SITS by the liver.

Whether the entry of SITS into the liver occurs by a saturable mechanism or not, is not clear. The 'initial' rate of uptake increases with the infusion rate and appears to saturate at higher rates of infusion (Fig. 2*A*), though not unequivocally. What is clear from Fig. 2*A* is that the uptake of SITS is not as good as the uptake of BSP. At the lowest rate of infusion used, the percentage extraction of SITS from the perfusate was only 36% as against 95% for BSP.

It is generally believed that the uptake of BSP into the liver cell occurs by a saturable mechanism (Reichen & Paumgartner, 1980). In view of this, and of the suggestion of saturation in SITS uptake in Fig. 2*A*, the data given in this Figure were analysed by means of a Lineweaver–Burk reciprocal plot in order to provide estimates of V and of K_m for comparison with other work. From this analysis the maximum rate of uptake (V) of both substances appeared to be similar (SITS, 0·15 μmol/min per g liver; BSP, 0·11 μmol/min per g liver) but the affinity of SITS for uptake was less than that of BSP (K_m for SITS $\equiv 8{\cdot}6 \times 10^{-5}$ M; K_m for BSP $\equiv 1{\cdot}8 \times 10^{-5}$ M).

Hepatic excretion

Under 'steady-state' conditions 56% of the SITS taken up by the liver may be accounted for by that excreted in bile (Fig. 2*B* and *C*). Allowing that the amount of SITS in bile may be underestimated by about 20% (as discussed in the Methods section), the true figure may be nearer 70%. Biliary excretion is clearly a major route of disposal of SITS by the liver. However, at least 30% of the SITS taken up under 'steady-state' conditions must be accumulated or disposed of within the liver by other means. The maximum rate of excretion of SITS in bile in these experiments was about 25 nmol/min per g liver which is roughly twice that obtained for BSP (10–16 nmol/min per g liver). Despite the low excretory rate for BSP, the hepatic uptake of this substance is well maintained (Fig. 2*B* and *C*), indicating that efficient alternative mechanisms exist for the disposal of BSP once it has entered the liver cell. The excretion of both SITS and BSP in bile occurs against a considerable concentration gradient: the bile/perfusate concentration ratios ranged from 50 to 170 for SITS and from 60 to 330 for BSP.

The decline in biliary excretion after the end of drug infusion differs significantly

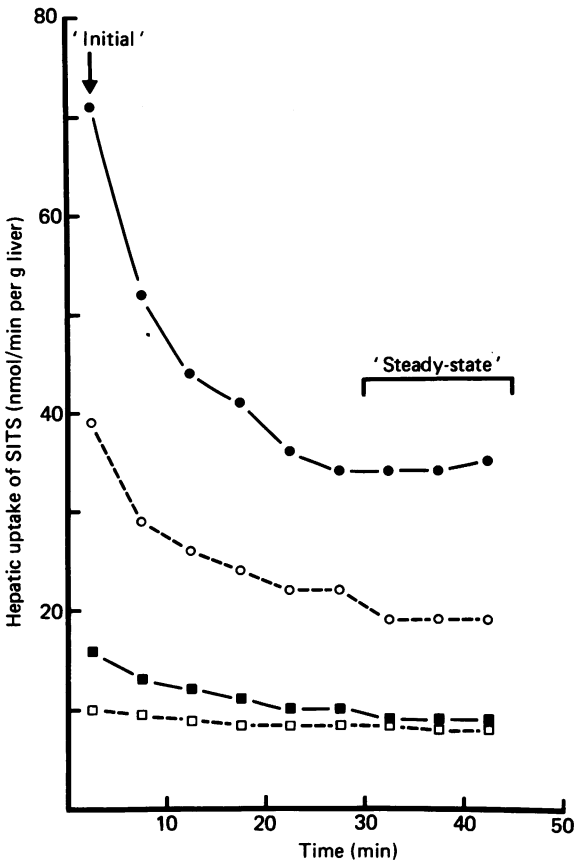

Fig. 1. Hepatic uptake of SITS during 45 min of perfusion with solutions containing SITS at various concentrations (●, 10^{-4} M; ○, 4×10^{-5} M; ■, 10^{-5} M; □, 5×10^{-6} M). Livers were perfused in a single-pass perfusion system with a Krebs–Ringer bicarbonate buffer (50 ml/min). Secretin was infused in all experiments except that marked with (○). The periods designated 'initial' and 'steady-state' in later Figures are defined.

for BSP and for SITS (Fig. 3). The biliary excretion of SITS falls off rapidly and exponentially in a manner which is consistent with it being derived from a single compartment. The excretion curve for BSP, however, is indicative of a more complex system which may involve two or more compartments, one of which perhaps acts as a store from which BSP may be recovered for excretion in bile.

SITS is excreted in bile in a form which is different to that infused. A number of compounds which fluoresced vividly under ultraviolet light were consistently found to be present in bile obtained during SITS infusion and not in the control bile samples (Fig. 4*A*). One of these had the same mobility as that of the infused SITS (*trans* form) but the identitiy of the remainder has not yet been determined. The relative contributions made by compounds migrating in specified bands of the chromatogram, to the total absorbance of bile at 340 nm is shown in Fig. 4*B*. A similar analysis made for chromatograms of standard solutions of *trans*-SITS is shown for comparison.

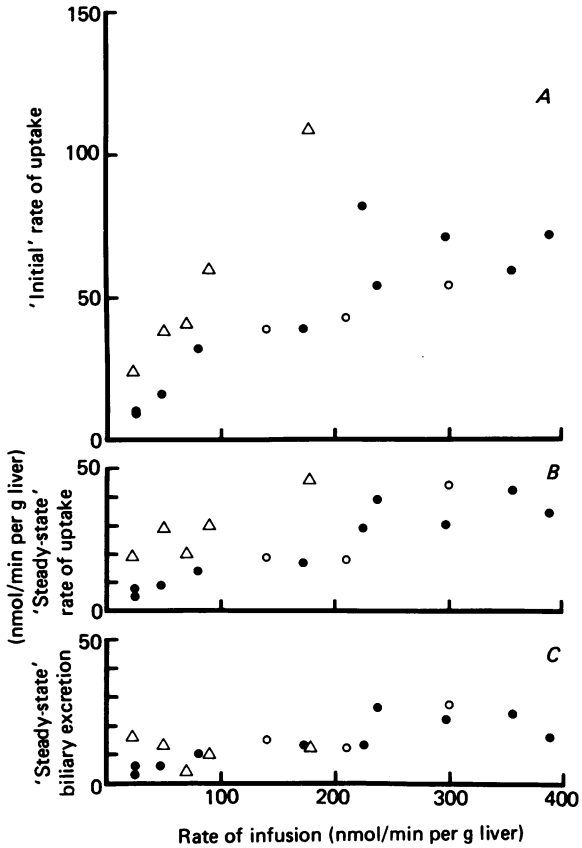

Fig. 2. Hepatic uptake and biliary excretion of SITS (●) and of BSP (△) at different infusion rates. *A*, 'initial' rate of uptake 1½–2 min after the onset of the infusion. *B*, mean rate of uptake in the 'steady-state' period 30–45 min after the start of infusion. *C*, mean biliary excretion in the 'steady-state' period 30–45 min after the start of infusion. Experimental conditions as in Fig. 1. Results from individual experiments are shown. ○, SITS in the absence of secretin.

Effects of SITS and BSP on bile flow

Both SITS and BSP decreased bile flow rate (Table 1). However, several differences were apparent: first, the concentration of BSP in the perfusate required to reduce bile flow was less than that for SITS; secondly, the effect of BSP occurred more rapidly; and thirdly the effect of BSP was readily reversed whereas that of SITS was not.

The time course of these effects, together with the differing patterns of uptake and excretion shown previously for SITS and BSP, suggested that the decrease in bile flow rate could be related to the amount of each substance which has accumulated in the liver. A statistically significant correlation was indeed found between the change in bile flow and the SITS content of the liver (Fig. 5*A*). A 50% decrease in bile flow is achieved at an estimated SITS content of about 1·5 μmol/g liver, which is equivalent to an intracellular concentration of about 2 mM. The content of BSP

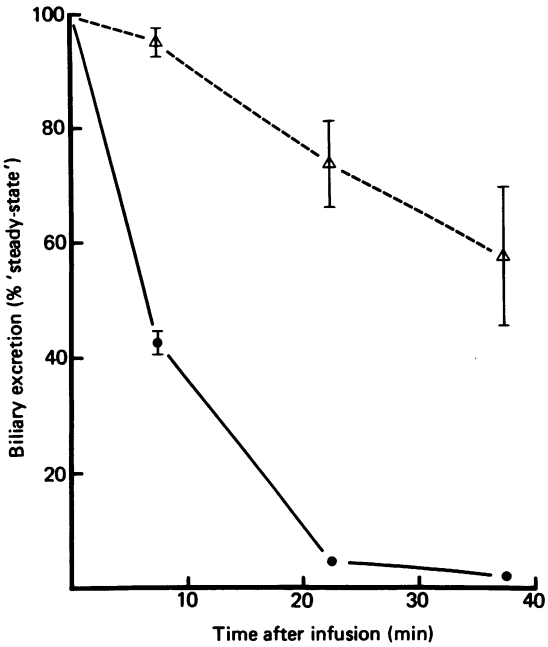

Fig. 3. The decrease in the biliary excretion of SITS (●) and of BSP (△) after the end of their infusion. Livers were perfused for 45 min with a Krebs–Ringer bicarbonate buffer containing either SITS or BSP. The infusion of SITS or BSP was then stopped but the perfusion continued and bile was collected as serial 15 min samples for a further 45 min. The excretion of SITS and of BSP in bile was expressed as a percentage of that obtained in the last 15 min infusion period. Mean ± S.E. of mean; $n = 3$. The calculated amounts of SITS and of BSP accumulated in the liver when the infusion was stopped were 1·11 ± 0·16 μmol/g for BSP and 0·98 ± 0·08 μmol/g for SITS (mean ± S.E. of mean; $n = 3$).

in liver required to produce the same decrease in bile flow is roughly the same (Fig. 5*A*). The reversibility of the effect of BSP fits well with the observation that BSP accumulated in the liver is recovered in large amounts in bile and in the liver effluent after the infusion of BSP is stopped. In *two* experiments the change in bile flow was correlated with the calculated BSP content of the liver at various times both during and after the infusion of the drug. Statistically significant linear correlations were obtained in each case ($r = 0{\cdot}91$. $P < 0{\cdot}02$; $r = 0{\cdot}99$, $P < 0{\cdot}001$; $n = 6$ in each experiment).

However, this correlation between bile flow and drug accumulation could be a consequence of the reduction in flow rather than its cause. Evidence against this view is presented in Fig. 5*B*. Here the initial rate of uptake of SITS into the liver is shown correlated with the resultant effect on bile flow after almost 45 min of infusion of the drug. A statistically significant correlation exists ($r = 0{\cdot}95$, $P < 0{\cdot}001$, $n = 10$) and it is evident that a decrease in bile flow is only produced when the initial rate of uptake of SITS exceeds 20–30 nmol/min per g liver. As this matches the maximum rate of excretion of SITS in bile found in these experiments, it strongly suggests that bile flow only decreases when the rate of hepatic uptake exceeds the maximum rate of biliary excretion.

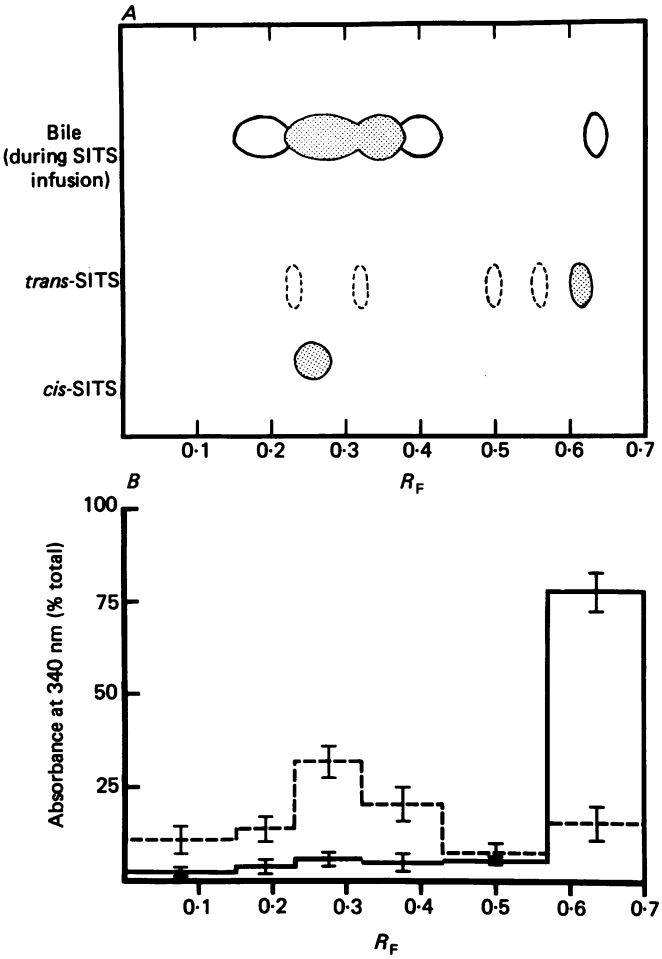

Fig. 4. Analysis of SITS in bile by thin-layer chromatography. *A*, diagram of a typical chromatogram showing (i) the spots observed under ultraviolet light when bile obtained during the infusion of SITS was analysed, (ii) the behaviour of *trans*- and *cis*-SITS in the solvent system. *B*, the relative absorbance at 340 nm (absorption maximum for *trans*-SITS) of water eluates of specified bands of the chromatograms shown in *A*: ----, bile obtained during the infusion of SITS; ——, *trans*-SITS. (Mean ± S.E. of mean; $n = 9$.)

Effects of SITS on the ionic composition of bile

The secretion of SITS in bile is associated with a decrease in the concentration of bicarbonate in bile and with an increase in the concentration of sodium. Both changes can be linearly related to the concentration of SITS in bile. For bicarbonate, $\Delta[HCO_3] = 0{\cdot}75\ [SITS] + 0{\cdot}68$; $r = 0{\cdot}81$; $P < 0{\cdot}0005$. For sodium, $\Delta[Na] = 0{\cdot}45\ [SITS] - 0{\cdot}45$; $r = 0{\cdot}76$; $P < 0{\cdot}01$. The concentrations of potassium and of chloride in bile did not change significantly.

The ionic composition of bile formed 45 min after the end of SITS infusion, when the excretion of SITS was very small, did not differ significantly from that in the control experiments, even though bile flow at this time was still significantly decreased.

TABLE 1. Bile flow as a percentage of control during and after 45 min infusions of either SITS or BSP or buffer alone

Infusate		Percentage bile flow: During infusion (A)	After infusion (B)	Net change (B − A)
SITS (M)	1×10^{-4}	52·1	38·0	−14·1
	1×10^{-4}	63·3	56·3	−7·0
	8×10^{-5}	78·4	63·1	−15·3
	4×10^{-5}	79·6	69·2	−10·4
	2×10^{-5}	75·2	57·3	−17·9
BSP (M)	4×10^{-5}	40·1	54·5	+14·4
	2×10^{-5}	55·3	67·9	+12·6
	2×10^{-5}	57·6	79·7	+22·1
	1×10^{-5}	71·5	96·0	+25·0
	5×10^{-6}	80·6	94·5	+14·5
Buffer alone		98·4 ± 9·2	84·0 ± 13·0	−14·4

The control period in each experiment was the 15 min bile sample preceding the infusion. 'During infusion': third 15 min bile sample during the infusion. 'After infusion': third 15 min bile sample after the infusion was stopped. Results for individual experiments are given, with the exception of those for 'buffer alone' (mean ± S.D. of an observation; $n = 6$).

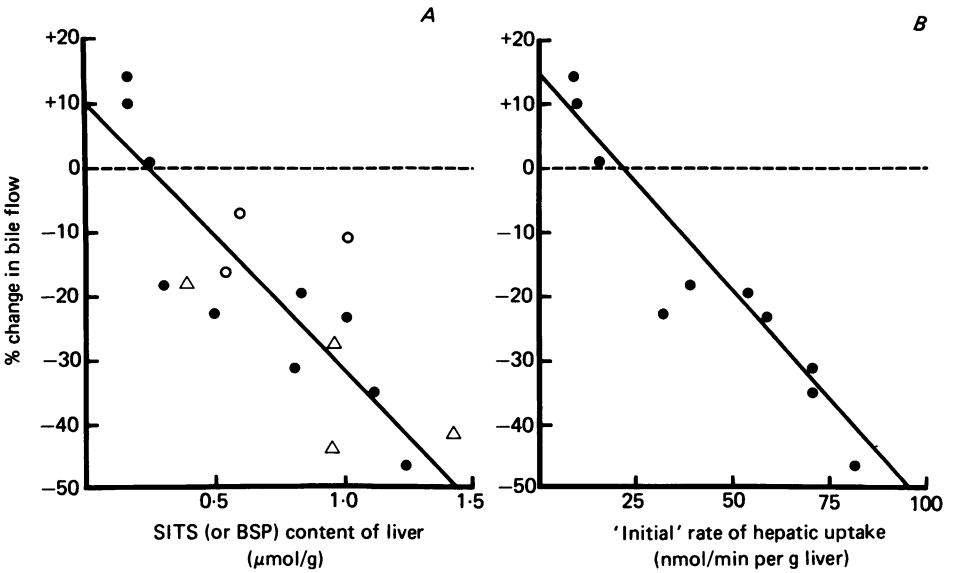

Fig. 5. The correlation between bile flow and variables related to the accumulation of SITS and of BSP within the liver. *A*, the percentage change in bile flow 30–45 min after the start of infusion, related to the calculated SITS (or BSP) content of the liver at that time. The regression line shown is that for ten experiments with SITS in the presence of secretin (●) ($y = 10{\cdot}0 - 42{\cdot}6x$, where y is the percentage change in bile flow and x is the calculated SITS content of the liver; $r = 0{\cdot}89$, $P < 0{\cdot}001$). BSP in the presence of secretin (△); SITS in the absence of secretin (○). *B*, The percentage change in bile flow 30–45 min after the start of infusion related to the 'initial' rate of uptake of SITS into the liver. (The equation of the regression line is: $y = 13{\cdot}5 - 0{\cdot}69x$, where y is the percentage change in bile flow and x, is the initial rate of uptake of SITS; $r = 0{\cdot}95$, $n = 10$, $P < 0{\cdot}001$).

Uptake, excretion and action of SITS in the absence of secretin

The results of three experiments are indicated in the relevant Figures (○, Figs. 1, 2 and 5). No differences were apparent in the uptake and excretion of SITS in bile. The effects of SITS on the flow and composition of bile were also essentially the same in the presence or absence of secretin, with the reservation that the effects of SITS on the rate of spontaneous secretion did not seem to be quite as great.

Liver histology. Pieces of liver removed during the infusion of SITS were fixed, sectioned and prepared by conventional techniques, mounted in Fluoromount and then examined under ultraviolet light. The cytoplasm of the parenchymal cells exhibited a marked, uniformly distributed fluorescence, whereas the nuclei of the same cells appeared dark. This prominent fluorescence of the parenchymal cell cytoplasm was not seen in liver sections which had not been exposed to SITS.

DISCUSSION

Hepatic transport of SITS

Like many other organic anions of similar molecular weight and chemical properties, SITS is taken up by liver cells, processed and excreted in bile. The maximum rate of uptake is similar to that for BSP (0·10–0·15 μmol/min per g liver) but the affinity of SITS for the uptake mechanism is clearly less by a factor of about five. The way in which organic anions gain entry into the liver cell is not yet clearly defined. Anions such as BSP bind to liver plasma membrane fractions (Cornelius, Ben-Ezzer & Arias, 1967; Reichen, Blitzer & Berk, 1981) and at least two proteins which bind BSP have been isolated from hepatocyte plasma membranes (Tiribelli, Lunazzi, Luciani, Panfili, Gazzin, Liut, Sandri & Sottocasa, 1978; Wolkoff & Chung, 1980). However, the role of carrier proteins in uptake has been disputed (Schwenk, Burr, Schwarz & Pfaff, 1976). It will be of interest to see whether these membrane fractions and isolated proteins are also capable of binding SITS and, if so, whether the dissociation constants *in vitro* match the relative affinities of SITS and BSP for the uptake mechanism observed in intact cells.

Having entered the liver cell, organic anions generally undergo conjugation with a more polar molecule and this is likely to be true also for SITS. Certainly, SITS is excreted in bile in a form which is different from that infused and this could well be a mixture of conjugates. Another stilbene derivative, *trans*-stilbene oxide, is a potent inducer of cytoplasmic glutathione-S-transferases (Guthenberg, Morgenstern, DePierre & Mannervik, 1980) and of microsomal UDP-glucuronyl transferase (Elmamlouk & Mukhtar, 1979), so that both glutathione and glucuronic acid could be likely candidates for conjugation with SITS.

The characteristics of SITS excretion in bile are similar to those for other organic anions. Excretion occurs against considerable concentration gradients and the maximum rate of excretion (25 nmol/min per g liver) is of the same order of magnitude as that for BSP (15 nmol/min per g liver). The maximum rate of excretion of BSP in bile reported here is only 50 % of that found in guinea-pigs *in vivo* (Whelan & Combes, 1971) but is similar to that reported in glutathione-depleted animals (Whelan, 1980). This may indicate some glutathione depletion in the perfused liver

preparation, promoted perhaps by the use of urethane, a known depleter of hepatic glutathione, as anaesthetic (Schulze & Czok, 1974). If SITS is conjugated with glutathione, depletion of this substance may alter the excretory capacity of the liver for SITS in the same way that it does for BSP. The maximum rate of excretion for SITS quoted here may therefore be an underestimate of normal capacity.

Both SITS and BSP can be accumulated in the liver at rates considerably in excess of their maximum excretion rate in bile. This indicates the existence of a 'storage' compartment within the liver into which the drug may be passed, thus maintaining hepatic uptake. Kinetic models for hepatic transport which include such a compartment have been described (Powell, Jones & Curtis, 1980). BSP seems to be readily released from this store, as judged by the recovery of BSP in bile at the end of infusion, whereas this appears not to be so for SITS. SITS accumulated within the liver could be broken down into products which are not detected by the methods of analysis used. Alternatively, SITS may be bound more firmly to intracellular ligands than is BSP and this in turn may explain the irreversible inhibition of bile flow produced by SITS.

Entry of SITS into cells

SITS cannot penetrate the red cell plasma membrane (Cabantchik, Knauf & Rothstein, 1978), a fact that has formed the basis of the assumption that this is true of other cells. The results of this study provide strong evidence that SITS is able to penetrate the membrane of the hepatocyte. Unless it be argued that SITS is handled differently from other organic anions which are efficiently excreted in bile, it is hard to escape the conclusion that SITS must have entered the liver cell in order to be excreted. Indeed, this conclusion is supported by the histological appearance of liver sections under ultraviolet light.

The liver, with its efficient mechanisms for the uptake of many organic anions, may therefore represent the opposite extreme within the body to the impenetrability of the red cell. Other cells which are known to possess transport systems for organic anions, such as those of the proximal convoluted tubule of the kidney and those of the intestinal epithelium (Lauterbach, 1975), may fall somewhere in between. SITS has been shown to inhibit the uptake of one organic anion, *p*-aminohippuric acid, into kidney cells, which of itself indicates an interaction of SITS with the carrier mechanism and possibly even some slight transport (Hong, Goldinger, Song, Koschier & Lee, 1978; Eveloff, Kinne & Kintner, 1979). Also, a related stilbene derivative, H_2DIDS, has been shown to be transported in small amounts into the proximal tubular lumen (Koschier, Stokols, Goldinger, Acara & Hong, 1980).

Inhibitory effects of SITS and BSP on bile secretion

The inhibitory effects of both SITS and BSP on bile flow correlate well with variables which are related to the accumulation of these drugs within the liver. This suggests that their locus of action may be intracellular. As the uptake of organic anions is believed to be a specific function of the parenchymal cells, it is likely to be these cells and not the ductular cells of the biliary system which are involved.

It has been shown *in vitro* that SITS can interfere with a variety of metabolic systems which exist within the cell, including Na^+–K^+-ATPase, carbonic anhydrase (Ehrenspeck & Brodsky, 1976) and the translocation of glucose-6-phosphate

across microsomal membranes (Zoccoli & Karnovsky, 1980). Inhibition of any one of these could interfere directly or indirectly with the process of bile secretion. Inhibitors of Na^+–K^+-ATPase have been shown both to increase and decrease the rate of bile flow. The role of Na^+–K^+-ATPase in the mechanism of bile secretion is, in consequence, still unclear (Reichen & Paumgartner, 1980). Inhibitors of carbonic anhydrase are known to decrease the choleretic effect of secretin in various species including the guinea-pig (Waitman, Dyck & Janowitz, 1969; Borjian & Rutishauser, 1982). However, the results described here are not entirely consistent with inhibition of this enzyme, as reciprocal changes in the concentrations of chloride and of bicarbonate in bile would be expected (Borjian & Rutishauser, 1982) but were not seen.

The anti-choleretic effect of BSP was first reported by Priestly & Plaa (1970) and has since been the subject of a number of studies. BSP has been shown to inhibit mitochondrial respiration and decrease the transfer of anions such as phosphate across mitochondrial membranes (Killenberg & Hoppel, 1974; Burr, Schwenk & Pfaff, 1977*a*, *b*). This leads to depletion of cellular ATP which may cause the associated inhibition of bile secretion (Laperche & Oudea, 1976). As a potent inhibitor of anion transport, SITS might also be expected to inhibit the translocation of anions across mitochondrial membranes and thus impair mitochondrial function. When Marrinetti & Gray (1967) examined the distribution of SITS in various cell fractions obtained from perfused livers or isolated cells exposed to a 10^{-3} M solution of SITS, they found that the greatest amount of SITS was associated with the mitochondrial fraction, with a lesser amount bound to the microsomes. This observation, which at the time proved inexplicable, may now provide an important clue as to at least one likely site of action of SITS within the cell. It may be that the effects of both SITS and BSP on bile secretion are caused, at least in part, by impairment of mitochondrial function.

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